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SPECIFICALLY DURING CARDIOVASCULAR STRESS
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**EFFECTS OF SIMULATED WEIGHTLESSNESS ON
REGIONAL BLOOD FLOW SPECIFICALLY DURING
CARDIOVASCULAR STRESS**

FINAL REPORT

Donald C. Harrison, MD
Principal Investigator

Division of Cardiology
Stanford University
Stanford, California

**Final Report: Effects of Simulated Weightlessness
On Regional Blood Flow Specifically
During Cardiovascular Stress**

Significant changes in the cardiovascular system of humans and animals have been observed following exposure to prolonged periods of weightlessness during space flight. Although adaption to weightlessness is relatively uncomplicated, marked changes in cardiovascular deconditioning become evident upon return to normal gravity, including orthostatic hypotension and tachycardia. Some evidence that myocardial degeneration occurs has been demonstrated in animals who have been immobilized for two months. Also, evidence of possible loss of myocardial mass following manned space flight has been obtained by means of echocardiographic studies.

These findings have serious implications in light of the increasing frequency and duration of Space Shuttle missions and the prospect of extended space station missions in the future. A number of both military and civilian investigators, including middle-aged scientists, will probably encounter prolonged periods of weightlessness. It has been imperative, therefore, to determine the effects of prolonged weightlessness on cardiovascular deconditioning and whether such effects are cumulative or reversible questions.

The research project conducted under NASA Cooperative Agreement NCC 2-126 was undertaken to determine the effects of prolonged simulated weightlessness on regional blood flow. Research results are reported in the following publications which are incorporated here for reference:

1. Clusin, W.T.; Buchbinder, M.; Ellis, A.K.; Kernoff, R.S.; Giacomini, J.C.; Harrison, D.C.: Reduction of Ischemic Depolarization by the Calcium Channel Blocker Diltiazem. Correlation with Improvement of Ventricular Conduction and Early Arrhythmias in the Dog. *Circulation Research* 54(1): 10-20, January 1984.
2. Clusin, W.T.; Buchbinder, M.; Ellis, A.K.; Kernoff, R.S.; Giacomini, J.C.; Harrison, D.C.: Reduction of Ischemic Depolarization by Diltiazem: A Flow-Independent Effect. *Circulation* 68(2): III-22, 1983.
3. Ellis, A.K.; Giacomini, J.C.; Kernoff, R.S.; Harrison, D.C.: Effects of Increased Right Ventricular Pressure on Coronary Blood Flow Distribution. *Federation Proceedings* 42(4): 1002, 1983.

Reduction of Ischemic Depolarization by the Calcium Channel Blocker Diltiazem

Correlation with Improvement of Ventricular Conduction and Early Arrhythmias in the Dog

William T. Clusin, Maurice Buchbinder, Avery K. Ellis, Robert S. Kernoff, John C. Giacomini, and Donald C. Harrison

From the Division of Cardiology, Stanford University School of Medicine, Stanford, California

SUMMARY. Calcium channel blockers suppress early ischemic arrhythmias, possibly by diminishing intracellular calcium overload and its effects on the ventricular action potential. To explore this, we compared the effects of diltiazem on ischemic "injury" potentials and ventricular fibrillation during serial coronary artery occlusions in dogs. Injury potentials and ventricular fibrillation were elicited every 15–25 minutes by simultaneous occlusion of the left anterior descending and circumflex arteries during rapid atrial pacing. DC epicardial electrograms were recorded differentially between the ischemic region and a small nonischemic region supplied by a proximal branch of the left anterior descending artery. Injury potentials developed with a uniform time course during five control occlusions, but were reduced by diltiazem infusion (0.5 mg/kg over 25 minutes) in each of eight dogs. The mean diastolic injury potential (T-Q depression) at 150 seconds of ischemia was 9.1 ± 2.7 mV before diltiazem and 6.1 ± 1.6 mV afterward ($P < 0.001$). Diltiazem increased the mean time between coronary occlusion and ventricular fibrillation from 186 to 366 seconds ($P < 10^{-5}$), but did not change the magnitude of the diastolic injury potential at onset of ventricular fibrillation. Diltiazem also delayed ischemia-induced conduction impairment to the same extent that it delayed injury potential development. In five dogs, the effect of diltiazem on regional blood flow near the epicardial electrodes was measured by infusion of radionuclide-labeled microspheres. Coronary occlusion reduced flow to the ischemic zone from 0.86 to 0.05 ml/min per g ($P = 0.001$). Diltiazem increased preocclusion flow by 11% ($P = 0.03$), but did not significantly alter flow during occlusion. Hemodynamic measurements show that diltiazem did not diminish cardiac work. Diltiazem therefore produced a flow-independent reduction of cellular depolarization during ischemia, which may be due to relief of calcium overload, and which may explain the antifibrillatory effect. (*Circ Res* 54: 10–20, 1984)

AN important property of calcium channel blockers is their ability to delay or prevent early ischemic arrhythmias during coronary artery occlusion. This phenomenon was first described by Kaumann and Aramendia in 1968, and has been confirmed in subsequent studies involving verapamil (Huang and Peng, 1977; Elharrar et al., 1977; Fondacaro et al., 1978; Brooks et al., 1980; Thandroyen, 1982), D-600 (Schoeneberger et al., 1979), diltiazem (Clusin et al., 1982; Thandroyen, 1982), nifedipine (Thandroyen, 1982), and several other dihydropyridines (Fagbemi and Parratt, 1981). The effects of calcium channel blockers on ischemic arrhythmias may shed light on the mechanisms by which these arrhythmias arise. Inward current carried by calcium has been suspected of mediating arrhythmias for many years (Cranefield, 1975). More recently, abnormal elevation of intracellular calcium has been shown to depolarize cardiac cells through direct effects on membrane permeability (Kass et al., 1978a, 1978b; Colquhoun et al., 1981; Matsuda et al., 1982; Clusin,

1983a, 1983b). Like older anti-anginal agents, calcium channel blockers reduce the "injury" currents, that are probably involved in initiating ischemic arrhythmias (Katzung et al., 1975; Kleber et al., 1978; Janse et al., 1980). This effect is usually ascribed to a decrease in the extent or severity of ischemia as a result of increased coronary perfusion, or reduced expenditure of energy as mechanical work. However, calcium channel blockers would also reduce injury currents if the effects of ischemia on the ventricular resting or action potential were a direct consequence of intracellular calcium overload (Clusin et al., 1983).

The study of arrhythmias is simplified if they can be elicited reproducibly during multiple trials in the same animal. We have previously found that simultaneous occlusion of the proximal LAD and circumflex arteries of the dog during rapid pacing induces ventricular fibrillation (VF) swiftly and predictably during successive trials (Clusin et al., 1982). The time between vessel occlusion and the onset of VF—

the VF latency—remains constant during as many as 15 consecutive control occlusions, but is markedly increased by infusion of diltiazem. This effect appears to be a direct result of reduced calcium influx into myocardial cells, because there is no associated increase in postocclusion coronary venous flow, and no reduction of left ventricular work. VF latency is also increased by infusion of sodium citrate, which reduces serum ionized calcium.

Through a simple modification of the above technique, we can now correlate arrhythmia onset with ischemia-induced changes in the ventricular action potential and conduction. This is accomplished by recording epicardial electrograms differentially between the ischemic portion of the left ventricle, and a small region that deliberately is not made ischemic. We obtain stable, high-amplitude records of ischemia-induced T-Q depression and S-T elevation, which has previously been possible only in small infarcts, where arrhythmias are not reproducible. Ischemic injury potentials and conduction impairment develop with a uniform time course during successive control occlusions, but their appearance is markedly slowed by diltiazem. These effects are closely correlated with changes in VF latency, and are not due to an increase in collateral coronary perfusion. The beneficial electrophysiological effects of calcium channel blockade may therefore be directly ascribable to reduction of intracellular calcium overload.

Methods

Healthy fasting mongrel dogs weighing 18–29 kg were sedated with morphine sulfate (2 mg/kg, intramuscularly) and anesthetized with intravenous chloralose (85 mg/kg) and urethane (625 mg/kg). Ventilation was provided by a Harvard pump, which delivered room air through a cuffed endotracheal tube. Aortic blood pressure was measured by a Statham P23Db transducer connected to a large-bore catheter inserted via the left femoral artery. A second catheter was placed in the left internal jugular vein for administration of additional anesthetics and experimental drug. Depth and rate of respiration were adjusted to maintain arterial pH between 7.35 and 7.45, P_{O_2} between 80 and 110 mm Hg, and P_{CO_2} between 35 and 45 mm Hg.

The heart was exposed through an incision in the left 4th intercostal space. A bipolar hook electrode was attached to the right atrium and connected to a WPI 800 series stimulator which delivered 2-msec current pulses at twice diastolic threshold. The proximal left anterior descending (LAD) and circumflex coronary arteries were exposed by dissection. Simultaneous reversible occlusion of these arteries was performed every 15–25 minutes, using nylon snares. The circumflex artery was occluded within 1 cm of its origin, while the LAD was occluded just beyond its first diagonal branch, leaving a small nonischemic region (perfused zone). All occlusions were performed during atrial pacing at a rate of 180–220 beats/min. Data collection began with the second occlusion, so that the preocclusion hemodynamics would be comparable during successive control trials (Clusin et al., 1982). When VF occurred, pacing was discontinued, both snares

were released, cardiac massage was instituted for 1 minute, and the animal was defibrillated using a 40–100 J pulse supplied by a Hewlett-Packard model 780 2C defibrillator with 5-cm paddles. The coronary snares were also released after several trials in which VF failed to occur within an arbitrary time limit of 420 seconds, owing to prior infusion of diltiazem. In rare instances, experiments were aborted because VF failed to occur within 240 seconds during the control trials.

DC epicardial electrograms were recorded using a pair of Ag/AgCl ECG electrode pellets (IMI, Inc.), that were sutured to the heart through holes drilled in their plastic casings, and connected to the inputs of a differential amplifier. Each pellet was 5 mm in diameter, and was coated with NaCl-containing electrode jelly (Hewlett-Packard REDUX paste). The negative (reference) electrode was sewn to the small region of myocardium supplied by the nonoccluded diagonal branch of the LAD, while the positive electrode was placed in the distribution of the occluded arteries, usually on the left ventricular free wall, near the apex. The electrodes were therefore 4–6 cm apart, with the positive electrode lying at least 2 cm from the perfused zone during coronary occlusion. The electrograms were filtered by a 1000 Hz, low-pass active filter, and drifted less than 300 μ V/5 min in the absence of ischemia. The electrograms recorded during ischemia were similar in amplitude and morphology to those obtained in previous studies involving unipolar recording (Kleber et al., 1978; Janse et al., 1980). However, in our experiments, placement of the reference electrode at an extra-cardiac site gave injury potentials much lower in amplitude and of variable morphology. This is presumably because the nonischemic zone was too small to drive electrically the remainder of the body, as it would in conventional regional ischemia, where the infarct is much smaller. The epicardial electrogram was recorded, together with aortic pressure and a standard electrocardiographic lead (II), by an optical strip chart recorder (Gould ES-100) at paper speeds of 25–200 mm/sec. The epicardial electrograms were displayed so that a positive deflection of the ischemic zone electrode with respect to the reference (perfused zone) electrode appeared upright.

Multiple control occlusions were performed in each dog before drug infusion. Diltiazem (d-3-acetoxy-cis-2,3-dihydro-5-(2-dimethylamino)ethyl)-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride; Marion Laboratories) was diluted in 0.9% NaCl to a concentration of 0.5 to 1.5 mg/ml and administered at a dose of 0.02 mg/kg per min for 25 minutes by a Harvard continuous infusion pump. A 25-minute infusion at this rate was previously shown to produce a mean serum diltiazem concentration of 173 ± 84 ng/ml, which declined exponentially ($\tau = 60$ min) when the infusion was terminated (Clusin et al., 1982).

In five dogs, coronary blood flow was measured by injection of radionuclide-labeled microspheres into the left atrium (Heymann et al., 1977). Four sets of microspheres, each bearing a different nuclide, were injected into each animal. Two sets were injected prior to diltiazem administration: one set 3 minutes before coronary occlusion and one set 30 seconds after occlusion. The other two sets were injected before and after an occlusion performed at the completion of the diltiazem infusion. Each injection contained approximately 30 million 8 to 10 μ m microspheres labeled with 125 I, 141 Ce, 51 Cr, 85 Sr, 95 Nb, or 46 Sc, and was completed over 20 seconds without significant

hemodynamic effect. A simultaneous timed blood collection of approximately 15 ml was made through the arterial catheter over a 90-second period beginning 5 seconds before each injection.

Hearts were excised after termination of the experiment and fixed for 3–5 days in 10% formalin. Two cylindrical transmural cores then were cut from the left ventricle, using a 2.5-cm (i.d.) sharpened steel cylinder that had an axial pin protruding beyond its cutting edge for centering. Each myocardial core was concentric with one of the epicardial electrode sites, and therefore included all of the myocardium that had been within 1 cm of that electrode. Each core was cut into four transmural layers; epicardial, endocardial, and two mid-myocardial. The four layers of each core were 2–5 mm thick, weighed at least 1.1 g, and contained a minimum of 100 microspheres of the nuclides administered before coronary occlusion. Radioactivity was counted with a 1024 channel γ well-counter (Packard model 9012). Regional blood flow was calculated on a digital computer by standard region-of-interest analysis (Heymann et al., 1977). Mean blood flow (in ml/min per g), was computed for each myocardial core, and for each of the four layers that comprised it.

Data from the above experiments have been displayed as mean \pm SD, except for the microsphere data, which were displayed as mean \pm SE, to conserve space. Mean VF latencies were computed using a value of 420 seconds for seven trials in which VF did not occur within this limit. Statistical comparisons involving two observations per dog were performed by paired *t*-test (two-tailed). Comparisons involving more than two observations were performed using the nonparametric Friedman test, which gives a χ^2 value for each analysis (Siegel, 1956). This test was especially suitable for the VF latency data because the effects of inter-animal variation were eliminated, and because the ranking procedure permitted simple handling of the trials in which VF did not occur.

Results

Reproducibility of Injury Potentials during Ischemia

The effects of ischemia on the DC epicardial electrogram are shown in Figure 1A. Ischemia produced a negative shift in the epicardial potential at QRS onset, which is sometimes called T-Q depression, and which we have called the diastolic injury potential, because an initial R wave was sometimes present. The diastolic injury potential was usually evident within 15 seconds of coronary occlusion and reached a mean value of 9.1 ± 2.7 mV after 150 seconds of ischemia ($n = 8$). True elevation of the T wave also occurred. Finally, ischemia reversed the polarity of the QRS complex, so that the predominantly negative deflection was replaced by a totally positive one. This phenomenon has been described previously (Kleber et al., 1978), and is due to delay or failure of action potential propagation into the ischemic myocardium (van Capelle and Janse, 1982).

The effects of ischemia on the electrograms developed with a characteristic time course, and were fully reversible when the coronary snares were released (Fig. 1B, left). The time course of the diastolic injury potential during a control occlusion has been

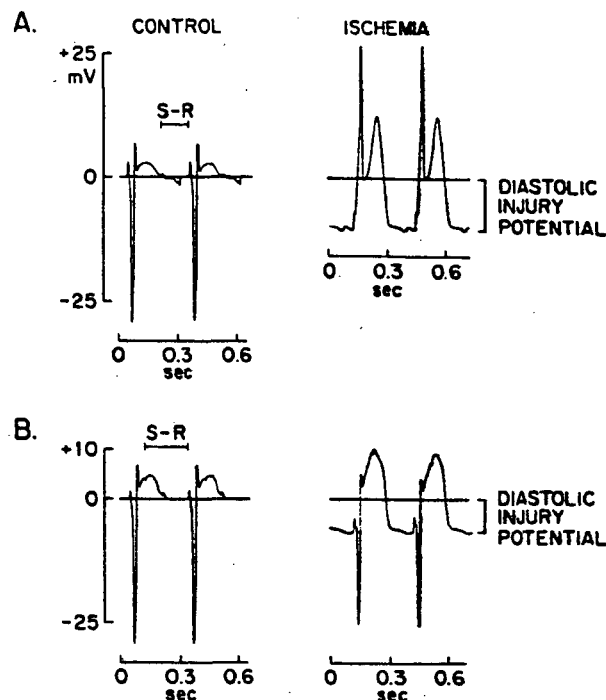


FIGURE 1. Effects of ischemia and diltiazem on the epicardial electrogram. Results shown in part A were obtained during a control occlusion, and in part B, after infusion of diltiazem. The positive electrode was placed within the ischemic zone, near the apex, while the reference electrode was placed over a small nonischemic region supplied by a diagonal branch of the LAD. Traces were obtained during right atrial pacing at 0 (left) and 135 seconds (right) of ischemia. Diltiazem reduced the ischemia-induced depression of the pre-QRS potential (diastolic injury potential), as well as the peak elevation of the T wave above the baseline. The change in QRS polarity from negative (downward) to positive (upward) was delayed by diltiazem, indicating relative improvement of conduction in the ischemic myocardium. In contrast, AV nodal conduction was slowed by diltiazem, as seen from the increased stimulus to R wave (S-R) interval.

plotted in Figure 2 (filled circles). Uniformity of the injury potentials during successive control occlusions, was demonstrated in two ways. In Figure 3, the diastolic injury potential has been averaged for six control occlusions in the same animal (filled circles). The relatively small standard deviations show that the injury potential at each point in time was similar during each occlusion. In the lower curve of Figure 5, the mean diastolic injury potential in eight dogs after 150 seconds of ischemia was plotted for each of five consecutive control occlusions, labeled C1–C5. The lack of significant variation during these occlusions was confirmed by the Friedman test ($\chi = 1.9$, $df = 4$, $P = 0.7$).

Effects of Diltiazem on Injury Potentials and Arrhythmias

Diltiazem reduced the ischemic injury potentials in every animal studied. Figure 1 compares epicardial electrograms obtained at 135 seconds during occlusions before (part A) and immediately after (part B) diltiazem infusion (0.5 mg/kg over 25 minutes). Diltiazem reduced the diastolic injury poten-

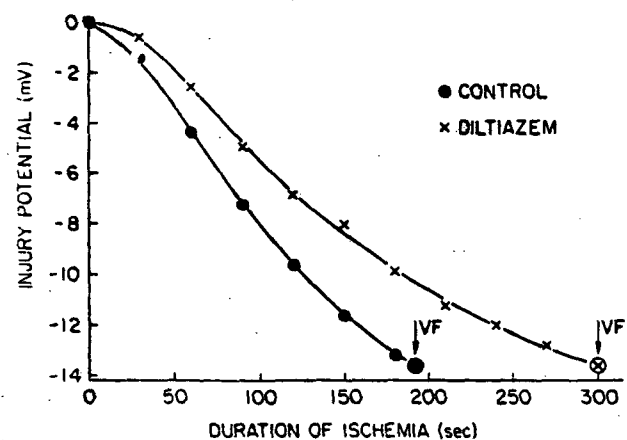


FIGURE 2. Effect of diltiazem on the time course of injury potential development. The diastolic injury potential (in mV) was measured at 30-second intervals, and plotted as a function of time during occlusions before (●) and immediately after (×) diltiazem. The injury potential was reduced by diltiazem at every point up to the development of VF (arrows). The magnitude of the injury potential at VF onset (circled symbols) was unchanged.

tial from 10.8 mV in part A to 7.0 mV in part B. The peak T wave elevation was also reduced, and atrio-ventricular conduction time (stimulus to R wave interval) was increased. As seen in Figure 2, diltiazem reduced the diastolic injury potential at every point in time up to the onset of VF. The injury potential increased again 1 hour after termination of the infusion (Fig. 3, open squares), when the serum diltiazem concentration would have declined e-fold (Clusin et al., 1982). Similar results were obtained in eight animals, giving the average data shown in

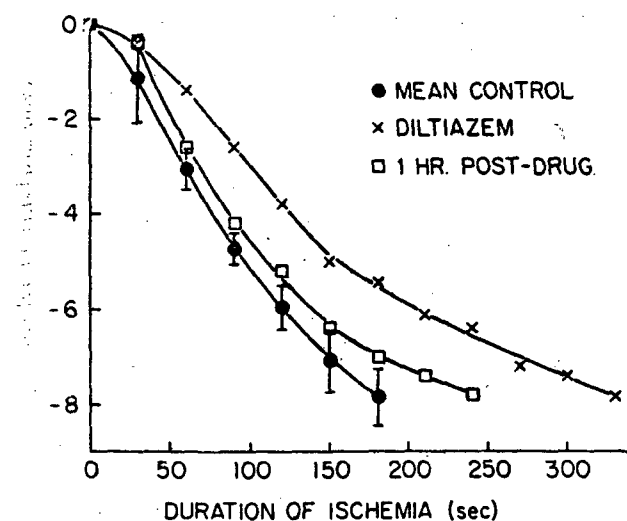


FIGURE 3. Injury potential development during multiple control trials and reversibility of the effect of diltiazem. The mean diastolic injury potential during six consecutive control occlusions has been plotted at 30-second intervals (●). The relatively small standard deviations (error bars) indicate the reproducible time course of injury potential development. The injury potentials were considerably smaller after diltiazem infusion (×) than the control values, but had increased substantially 1 hour later (□).

Figure 4. The mean diastolic injury potential was significantly reduced by diltiazem at every point in time ($P = 0.01$ to 0.004). Even greater statistical significance ($P = 0.01$ to 0.0003) was demonstrated when the injury currents from each animal were normalized (expressed as a percentage of the control value at 150 seconds).

Reduction of the diastolic injury potential by diltiazem was always associated with prevention of VF during the period of observation (up to 420 seconds) or with a delay in VF onset compared to the control trials. The mean VF latency before and after diltiazem infusion has been plotted in the upper curve of Figure 5. Diltiazem increased VF latency beyond the five control values in all eight dogs, and in four dogs, VF was prevented during the first post-drug trial (D1). Onset of less severe arrhythmias (ventricular premature beats and unsustained ventricular tachycardia) was also delayed by diltiazem, in agreement with the findings of Patterson et al. (1983). The changes in arrhythmia latency after diltiazem infusion were similar to the changes in diastolic injury potential (lower curve of Fig. 5). The mean diastolic injury potential at 150 seconds (lower curve of Fig. 5) was smaller after diltiazem infusion than during any of the five control trials, and was slightly smaller during the first post-drug trial (D1) than the

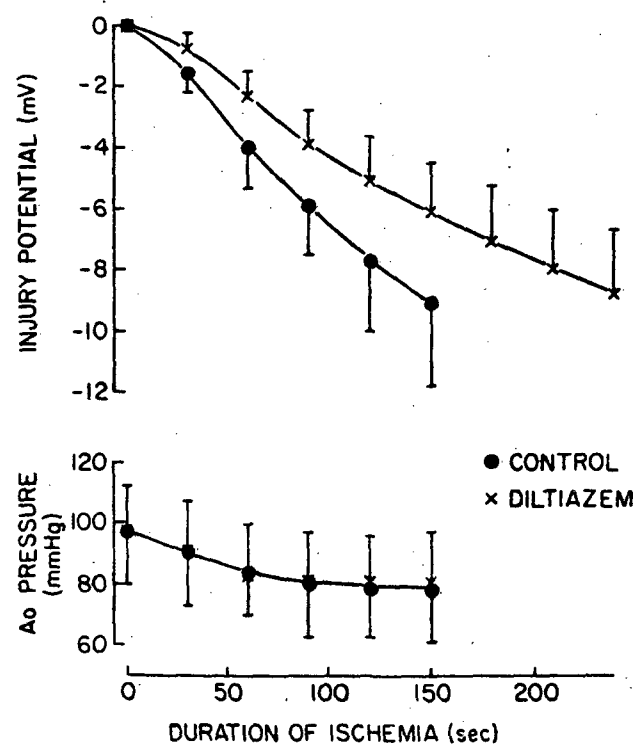


FIGURE 4. Mean diastolic injury potential and aortic systolic blood pressure in eight dogs during coronary occlusions before (●) and immediately after diltiazem (×). The mean diastolic injury potential (upper curves) was significantly larger during the control occlusions at every point in time. Coronary occlusion produced a similar decline in the mean aortic systolic pressure, before and after diltiazem (lower curves).

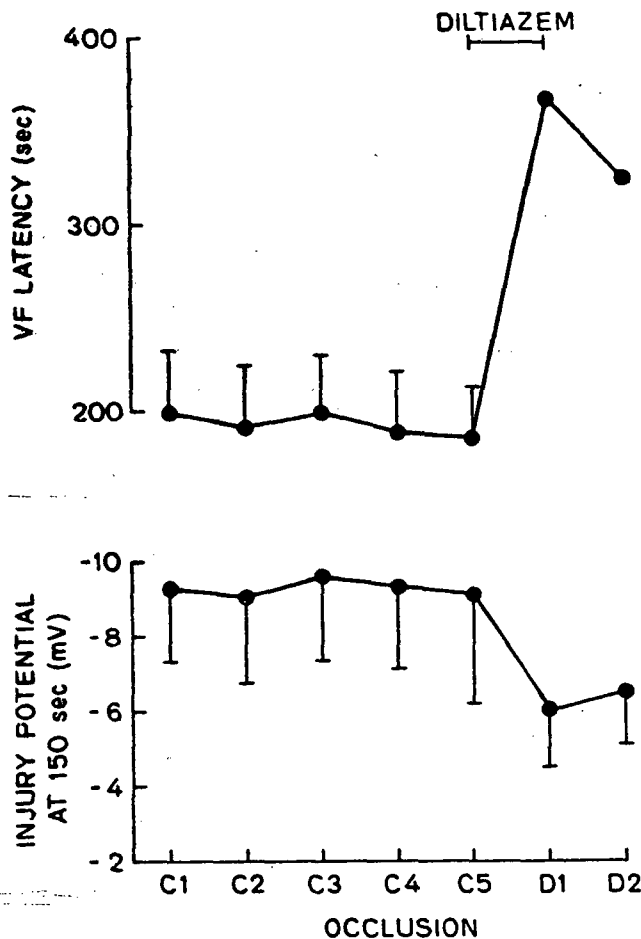


FIGURE 5. Mean VF latency and diastolic injury potential in eight dogs before and after diltiazem. Measurements were obtained during five control occlusions (C1-C5) and two occlusions after diltiazem infusion (D1-D2). Mean VF latency (upper curve) was relatively constant during the control trials, increased markedly after diltiazem, and declined somewhat during the second post-drug trial (D2). Error bars were omitted for the post-drug trials because VF sometimes failed to occur within the maximum observation period of 420 seconds. The mean diastolic injury potential at 150 seconds of ischemia was plotted for the same occlusions in the lower curve. The injury potential was markedly reduced immediately after diltiazem (D1) and somewhat less so during the second post-drug trial (D2).

second (D2). The statistical significance of these results was corroborated when all seven trials were analyzed by the Friedman test ($\chi^2 = 35.1$ for VF latency; 33.1 for injury potential, $df = 6$, $P < 10^{-5}$ for both curves).

Failure of Diltiazem to Restore Perfusion to the Ischemic Zone

Reduction of the diastolic injury potential by diltiazem could have resulted from drug-induced restitution of coronary perfusion near the ischemic zone electrode, or from a flow-independent reduction of cellular depolarization. The first possibility was intentionally minimized by the experimental design, and was directly tested in five of the eight dogs by infusion of radionuclide-labeled microspheres for

measurements of regional blood flow. The diameter of the myocardial specimens analyzed was 2 cm larger than the electrode diameter, and was also large, compared with the electrical space constant of ventricular muscle. Any myocardium which could have influenced the electrograms was therefore included in the blood flow measurements.

The effects of coronary occlusion and diltiazem on blood flow near the electrode sites are shown in Figure 6. Prior to diltiazem (filled circles), coronary occlusion reduced blood flow to the ischemic zone specimens for a basal level of 0.86 ± 0.10 ml/min per g to an insignificant level of 0.05 ± 0.02 ml/min per g ($P = 0.001$). A smaller reduction in flow occurred in the perfused zone specimens, due either to overlap of the specimens with ischemic myocardium, or to the decline in aortic blood pressure during ischemia. Diltiazem produced an 11% increase in blood flow to the ischemic zone specimens prior to coronary occlusion ($P = 0.03$), and a comparable increase in flow to the perfused zone specimens. However, there was no significant change in flow to the ischemic specimens subsequent to occlusion ($P = 0.4$).

Because extracellular electrodes are most sensitive to depolarization in nearby tissue, diltiazem might have diminished the injury potentials by causing redistribution of residual coronary flow toward the epicardium. This possibility was excluded by computation of flow to each of the four layers that comprised each myocardial specimen. After diltiazem

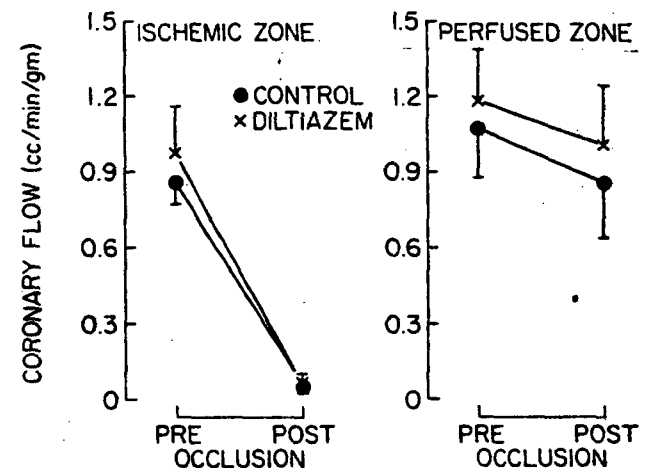


FIGURE 6. Effects of diltiazem and coronary occlusion on blood flow near the epicardial electrode sites in five dogs. Regional blood flow was calculated from measurements of microsphere-bound radioactivity in transmural cores centered around the ischemic zone (left) and perfused zone (right) electrode sites. Microspheres labeled with different nuclides were infused before and after coronary occlusion in the presence (X) and absence (●) of diltiazem. Coronary occlusion reduced flow in the ischemic zone nearly to zero, and produced a smaller reduction in the perfused zone. Diltiazem produced a small increase in flow to the perfused zone, and to the ischemic zone prior to coronary occlusion, but did not appreciably alter flow to the ischemic zone during occlusion.

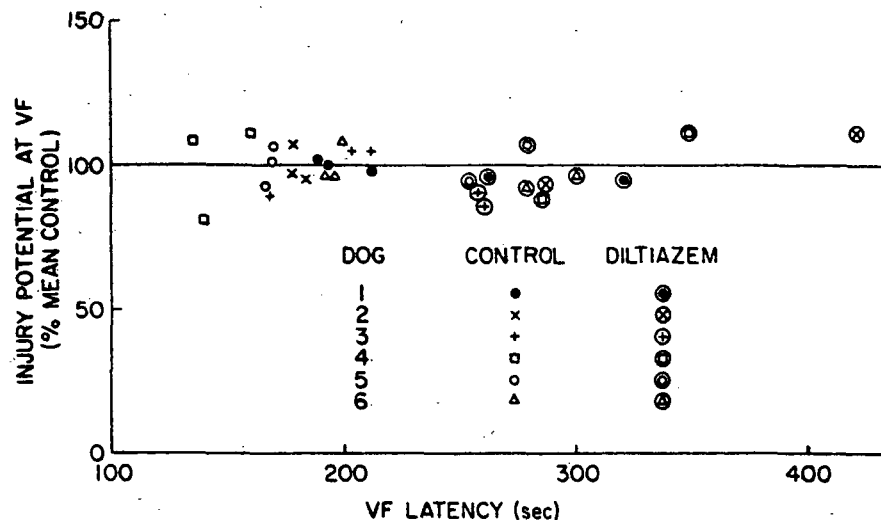


FIGURE 7. Failure of diltiazem to alter the injury potential at VF. The diastolic injury potential at VF onset was plotted on the vertical axis, and the corresponding duration of ischemia (VF latency) on the horizontal axis. The three control occlusions preceding drug infusion were compared with the first two post-drug occlusions in which VF occurred (circled symbols). Each injury potential was expressed as a percentage of the mean of the control values for that dog. VF was invariably delayed by diltiazem, but the injury potential at VF onset remained within the control range. Data are from the same dogs as in Figures 4-5, except that two dogs were excluded in which VF did not occur during the several occlusions after diltiazem infusion.

azem infusion, the postocclusion flow to the epicardial layer of the ischemic zone specimen was <0.05 ml/min per g in each of the five dogs (mean value = 0.03 ± 0.01 ml/min per g).

To confirm that the regional blood flow measurements were directly pertinent to the electrophysiological findings, the mean diastolic injury potential was computed for the subgroup of five animals in which blood flow was also measured. The normalized injury potentials were significantly smaller after diltiazem at every point in time ($P = 0.01$ to 0.003). Reduction of these potentials by diltiazem was therefore not due to increased perfusion near the ischemic zone electrode.

Constancy of the Injury Potential at VF Onset

Depolarization of ischemic myocardial cells is believed to cause ischemic arrhythmias directly, either by inducing ventricular automaticity (Katzung et al., 1975) or by producing sufficient slowing of conduction to permit reentry. If the increase in VF latency by diltiazem were due entirely to slowing of ischemia-induced depolarization, then the magnitude of the diastolic injury potential at VF onset would not be altered by the drug. This prediction was born out in Figure 2, where the diastolic injury potential at VF was identical before and after diltiazem. To test the generality of this observation, the diastolic injury potential was plotted as a function of VF latency for six dogs in which VF recurred after diltiazem infusion (Fig. 7). Injury potentials were normalized to the mean of the control values to facilitate comparison among dogs. Although the VF latency after diltiazem (circled symbols) always exceeded the control values, the diastolic injury poten-

tial at VF onset remained within the control range. The mean diastolic injury potential at VF was 10.0 ± 2.7 mV for the 18 control occlusions in Figure 7, compared with 9.6 ± 2.5 mV for the 12 occlusions performed after drug infusion ($P = 0.7$). Thus, the degree of depolarization near the ischemic zone electrode at the onset of VF was not significantly altered.

Effect of Diltiazem on the Ischemia-Induced Conduction Delay

The apparent relation between ischemia-induced depolarization and VF could reflect the fact that depolarization slows conduction within the ischemic zone by causing inactivation of fast sodium channels. Although the epicardial electrograms did not permit direct measurement of conduction velocity, the effect of ischemia on conduction could be inferred from the change in the morphology of the QRS complex. In every control recording, the initial QRS complexes contained a large negative deflection, which was abolished or greatly diminished after 150 seconds of ischemia, leaving a predominantly or totally positive deflection. In five of the eight dogs, the negative deflection was initially larger than any positive deflection, so that there was overall reversal of QRS polarity during ischemia (e.g., Fig. 1A). As noted above, the ischemic zone electrode was usually located near the LV apex, whereas the perfused zone electrode was on the free wall near the AV groove. The predominant negativity of the QRS complex prior to ischemia was therefore in accordance with the normal ventricular activation sequence, and its reversal during ischemia was an expected result of impaired conduction

within the ischemic zone (Kleber et al., 1978; van Capelle and Janse, 1982).

The changes in QRS morphology produced by ischemia were highly reproducible during successive control occlusions. In Figure 8, the amplitude of the largest QRS deflection (measured with respect to the pre-QRS potential) has been determined at 15-second intervals during five consecutive control occlusions, giving the average values plotted as filled circles. The relatively small standard deviations show that the changes in the QRS complex were similar during each occlusion. Diltiazem greatly delayed the effects of ischemia on ventricular conduction. In Figure 1B, the QRS complex after 135 seconds of ischemia was predominantly negative, even though it was totally positive in the control recording (Fig. 1A). As seen in Figure 8, reversal of the QRS polarity was not prevented by diltiazem, but was substantially delayed.

The effects of ischemia and diltiazem on ventricular conduction could be compared more simply by measuring the duration of coronary occlusion necessary to convert the QRS complex from predominantly negative (largest deflection negative) to predominantly positive. This time interval, which we have called the activation reversal latency, was determined for each of the five dogs in which a predominantly negative QRS was initially present, giving the mean values plotted in the upper curve of Figure 9. The mean activation reversal latency was nearly constant during the five control occlusions (C1-C5), but increased beyond the control range in

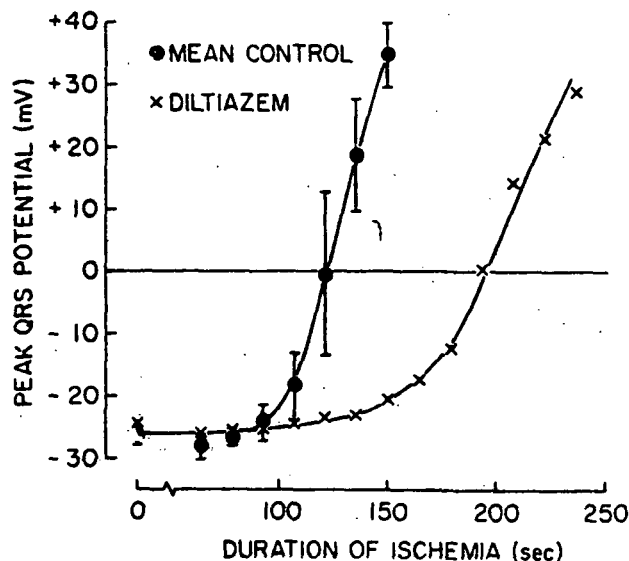


FIGURE 8. Changes in peak QRS potential during serial coronary occlusions before and after diltiazem. The largest deflection of the epicardial QRS complex was measured at 15-second intervals during six consecutive occlusions in the same dog. Mean values during five control occlusions were plotted as filled circles. The ischemia-induced reversal of QRS polarity occurred with a nearly uniform time course during the control trials, as shown by the standard deviations (error bars), but was markedly delayed by diltiazem (X). A value of zero was plotted at 195 seconds for the post-drug trial because the positive and negative QRS deflections were of equal amplitude.

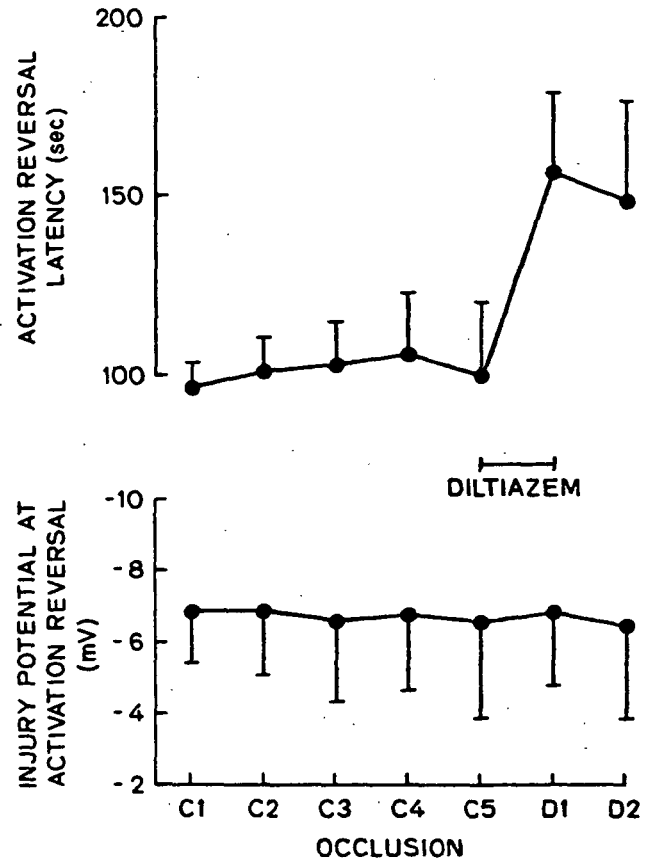


FIGURE 9. Effect of diltiazem on ischemia-induced QRS reversal (activation reversal). The duration of coronary occlusion at which the positive QRS deflection first exceeded the largest negative deflection was determined in five dogs whose preocclusion electrograms contained predominantly negative QRS complexes. Mean values were plotted in the upper curve for five control occlusions (C1-C5) and two occlusions after diltiazem (D1-D2). Activation reversal occurred at nearly constant latency during control trials, but was significantly delayed by diltiazem. The diastolic injury potential at the moment of activation reversal was also determined, giving the mean values in the lower curve. The injury potential at activation reversal was nearly constant before and after diltiazem.

each of the five dogs after diltiazem infusion. The changes in activation reversal latency were highly significant by the Friedman test ($\chi^2 = 20.7$, $df = 6$, $P = 0.002$). A separate analysis of the five control occlusions showed no significant variation ($\chi^2 = 3.6$, $df = 4$, $P = 0.5$).

If the preservation of normal ventricular conduction by diltiazem were purely a result of reduced depolarization, then the degree of depolarization needed to produce a pre-defined conduction impairment would not be altered by the drug. This relationship was studied by measuring the diastolic injury potential at the moment of activation reversal for each of the trials in Figure 9. Although injury potential development and activation reversal were both delayed by diltiazem, the mean injury potential at the time of activation reversal (lower curve) was virtually unchanged. The absence of significant variation during the seven occlusions was confirmed by the Friedman test ($\chi^2 = 0.01$, $df = 6$, $P = 0.99$).

Discussion

Early arrhythmias during coronary occlusion ultimately result from effects of ischemia on the ventricular resting and action potential. Rapid changes in transmembrane potential have been observed in intracellular recordings from infarcts (Kleber et al., 1978; Janse et al., 1980), and are faithfully reflected by the "injury" potentials simultaneously recorded from the epicardial surface. Van Capelle and Janse (1982) have examined the reproducibility of these phenomena in a small number of animals in which microelectrode impalements could be maintained during multiple occlusions. They find that the effects of ischemia on membrane potential occur with a reproducible time course during at least three consecutive trials.

In the present study, the uniform time course of ischemic injury potentials enables us to demonstrate reduction of these potentials by diltiazem, and to correlate this effect with delay or prevention of ventricular fibrillation. The injury potentials, and changes in QRS potential that we record are similar in amplitude and time course to those seen in conventional regional ischemia. However, our results have been obtained under conditions approaching global LV ischemia, where arrhythmia onset occurs predictably during brief ischemic episodes (Clusin et al., 1982). The key innovation which makes this possible is the use of a differential recording configuration that gives full-sized injury potentials, despite the extremely small size of the nonischemic region.

S-T Segments and Infarct Size

Improvement of ischemia-induced S-T changes by a drug is often equated with reduction of myocardial infarct size, although the validity of this inference has been questioned (Fozzard and Das Gupta, 1976). In our experiments, this interpretation has been excluded by measurement of residual blood flow over a region several electrical space constants larger than the epicardial electrodes. The residual flow to the ischemic myocardium before and after diltiazem is comparable to that measured in pig hearts, which lack intercoronary collaterals (Fujiwara et al., 1982), and is consistent with the profound reduction of coronary venous flow that we previously observed after combined LAD and circumflex artery occlusion (Clusin et al., 1982). In some dogs, residual flow near the ischemic zone electrode becomes undetectable during the coronary occlusions. The effects of diltiazem on the injury potentials are no different in these cases, than in dogs with a non-zero residual flow. Three features of our model presumably account for the low levels of residual coronary flow: (1) the brevity of the occlusions, (2) the small size of the perfused zone, and (3) deliberate placement of the ischemic electrode as far as possible from perfused myocardium.

Our results do not exclude possible enlargement of the nonischemic region by diltiazem. Such an effect would be consistent with the observed in-

crease in postocclusion flow near the perfused zone electrode (Fig. 6, right). However, because of the configuration of the recordings, an increase in perfusion near this electrode could only increase the amplitude of the injury potentials. The observed reduction of these potentials must therefore involve a direct effect of diltiazem on the ischemic myocardium.

The ability of calcium channel blockade to diminish the characteristic effects of ischemia through a flow-independent mechanism has been demonstrated in intracellular recordings from isolated dog ventricular strips during brief superfusions with hypoxic, potassium-rich, acidic saline (Kimura et al., 1982). In these experiments, the effects of the altered saline developed with a reproducible time course during successive trials in the same preparation. Pretreatment with verapamil caused relative preservation of epicardial action potential amplitude, upstroke velocity and conduction velocity at 5 and 10 minutes of simulated ischemia.

Metabolic Considerations

A second means by which diltiazem might slow the development of ischemic injury potentials is through reduction of energy expended as mechanical work. We have measured the hemodynamic effects on an identical diltiazem infusion, and find that, whereas the mean aortic pressure during ischemia is unchanged, the cardiac output increases significantly, presumably in response to peripheral vasodilation (Clusin et al., 1982). Thus, in our model, mechanical energy expenditure is increased rather than decreased by diltiazem. Although we did not measure cardiac output in the present study, the failure of diltiazem to alter aortic blood pressure (Fig. 4, lower curves) suggests that its effects on cardiac work were as previously found.

There is a variety of evidence that the "protective effect" of calcium channel blockers during ischemia need not be contingent upon an increase in myocardial energy stores. Jolly et al., (1981) found that the more complete recovery of contractile function in diltiazem-treated guinea pig hearts after ischemia is not associated with an increase in high energy phosphate stores during ischemia. Thandroyen (1982) found that the antifibrillatory effect of diltiazem could be observed in the absence of increased high energy phosphates. Finally, Henry and Wahl (1983) found that diltiazem doubles the duration of hypoxia needed to produce contracture in quiescent ventricular muscle. This protective effect is not due to reduction of systolic force, since no phasic contractions occur.

Does Calcium Overload Directly Affect Membrane Potential?

A third explanation for the reduction of ischemic depolarization by diltiazem is that depolarization occurs as a direct consequence of intracellular calcium overload, which develops rapidly during met-

abolic inhibition (Dahl and Isenberg, 1980), and ischemia (Katz and Reuter, 1979; Clusin et al., 1983). Cardiac cells are known to contain inward current channels that are opened exclusively by the presence of calcium ions at the inner surface of the cell membrane (Colquhoun et al., 1981). Moreover, extrusion of calcium across the surface membrane in exchange for sodium may involve net inward movement of positive charge (Mullins, 1979). These mechanisms are believed to produce the transient inward current (TI) of digitalis toxicity (Kass et al., 1978a, 1978b), which occurs when sequestered calcium is inappropriately released during diastole. A similar process may occur in ischemia, due to the energy requirement for calcium sequestration. Clusin (1983b) has shown that exposure of voltage-clamped cardiac cells to metabolic inhibitors elicits a depolarizing inward current which is suppressed by removal of external calcium, and has several other features in common with the digitalis-induced TI. A similar, though less sustained, depolarizing current is induced by 10 mM caffeine, which causes calcium release from the sarcoplasmic reticulum (Clusin, 1983a).

Calcium overload may also lead to shortening of the cardiac action potential during ischemia. Action potential shortening is observed in several conditions that increase intracellular calcium, including digitalis toxicity, increased beat frequency, increased extracellular calcium, and intracellular injection of calcium with microelectrodes (Isenberg, 1977; Kurachi, 1982). In digitalis toxicity, the shortening of the action potential can be reversed by intracellular injection of EGTA (Matsuda et al., 1982). A direct role of intracellular calcium would be consistent with our observation that diltiazem reduces true S-T elevation during ischemia, although changes in conduction might also affect T wave morphology in our experiments.

Prevention of calcium overload could diminish other effects of ischemia that can influence the resting and action potential. Increased extracellular potassium, for example, is at least partly responsible for the early reduction of the resting potential during coronary occlusion (Hill and Gettes, 1989; Kleber, 1983). Since calcium-activated channels in cardiac cells are permeable to potassium (Colquhoun et al., 1981), prevention of calcium overload could diminish potassium efflux during ischemia. Acidosis may also contribute to the electrophysiological effects of ischemia (Morena et al., 1980; Gilmour and Zipes, 1980; Kagiya et al., 1982). Kurachi (1982) has recently shown that the effects of intracellular proton injection on the action potential and membrane current of ventricular cells are similar to those of calcium injection. He ascribes this similarity to a reversible exchange of intracellular protons for sequestered calcium ions, which has also been proposed by Vaughan-Jones et al. (1983). Changes in the action potential during acidification could there-

fore be due, at least in part, to effects of liberated intracellular calcium on membrane permeability. Moreover, prevention of calcium overload by a drug might diminish cytoplasmic acidification during ischemia.

The Relation of Ischemic Depolarization to Conduction Changes and Arrhythmias

Diltiazem's ability to preserve normal action potential characteristics during ischemia may explain the prolongation of arrhythmia latency by this drug. There are two ways in which reduction of ischemic depolarization could retard arrhythmias. First, Katzung et al., (1975) suggest that ischemic depolarization and the resulting diastolic injury current induce ventricular automaticity. Their hypothesis is consistent with the fact that the first beat of ventricular fibrillation arises in normal myocardium near the ischemic boundary (Janse et al., 1980; Ideker et al., 1981). Since depolarization-induced automaticity occurs at a reproducible stimulus strength, this mechanism could explain why the diastolic injury potential at the onset of VF remains constant after diltiazem (Fig. 7). Alternatively, VF may be a consequence of slowed conduction within the ischemic zone. Reduction of ischemia-induced conduction delay has been demonstrated previously with verapamil (Elharrar et al., 1977), diltiazem (Peter et al., 1982), and nifedipine (Nakaya et al., 1981), and has been implicated in the suppression of early arrhythmias (Elharrar et al., 1977).

Improvement of conduction by calcium channel blockers is considered paradoxical because, in depolarized fibers, calcium channel blockade should impair conduction further. Four mechanisms could account for improvement of conduction by these drugs: (1) The improvement observed by Peter et al. (1981) may have been due to reduction of infarct size because conduction was improved by a relatively constant amount during 30 minutes of ischemia, and because the nonoccluded blood supply was extensive. This explanation is unlikely because, in our study there was negligible blood flow near the ischemic electrodes, and only short-term preservation of conduction. (2) Diltiazem might have improved conduction by causing reflex-mediated catecholamine release (Danilo et al., 1978). Although we have no evidence against this hypothesis, it probably is not the primary mechanism, since reduction of injury potentials by propranolol is accompanied by a similar improvement in conduction (Clusin and Buchbinder, unpublished observations). (3) Improved conduction could be due to increased sodium channel availability as a result of reduced depolarization. This mechanism undoubtedly accounts for at least some of the conduction improvement which we observe, because the diastolic injury potential was diminished at the same electrode sites where conduction was studied. (4) Diltiazem might improve conduction by causing a relative increase

in membrane resistance during ischemia. Hypoxia (Mascher and Carmeliet, 1975) and metabolic inhibitors (Clusin, 1983b) both decrease membrane resistance in cardiac cells, even when membrane potential is held constant. Ischemia could have similar effects, since it causes greater conduction delay than equivalent depolarization by potassium (Hill and Gettes, 1980; Morena et al., 1980). If the resistance decrease in the above conditions is mediated by calcium-activated ion channels (Clusin, 1983b), then prevention of calcium overload would necessarily improve conduction.

Can Preservation of Action Potential Characteristics Lead to Arrhythmia Prevention?

An important question raised by our observations is whether the slowing of ischemic depolarization by diltiazem would prevent VF or merely delay its onset. In dogs, lethal arrhythmias seldom occur more than 20 minutes after coronary occlusion (Kaplinsky et al., 1979). Later onset of VF may be prevented by electrical uncoupling (van Capelle and Janse, 1982) or by spontaneous improvement of action potential characteristics (Morena et al., 1980; Gilmour and Zipes, 1980). Whatever the reason for the limited period of vulnerability, preservation of the resting and action potential during this period could potentially prevent arrhythmias, even if no myocardium were ultimately salvaged.

Diltiazem rarely prevents VF during global LV ischemia, even though VF latency may increase several-fold (Clusin et al., 1982). In contrast, diltiazem can abolish VF when only the LAD is occluded (no VF in 6/6 dogs; Schnittger et al., unpublished data), as can verapamil (Kaumann and Aramendia, 1968). Since the majority of VF episodes produced by LAD occlusion in untreated dogs occur between 13 and 20 minutes of ischemia (Kaplinsky et al., 1979), a moderate increase in VF latency by diltiazem could, in this situation, cause the usual period of vulnerability to be exceeded.

It is uncertain whether similar protective effects occur in the clinical setting. In patients with angina, diltiazem, at serum concentrations similar to those used here, increases the duration of treadmill exercise needed to produce S-T segment depression (Hung et al., 1982), and increases the work load at which criterion S-T segment depression is achieved (Wagniart et al., 1982). This latter finding seems consistent with our conclusion that diltiazem retards the effects of ischemia on the action potential, even when the extent and severity of ischemia are unchanged.

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Address for reprints: Dr. William T. Clusin, Division of Cardiology, Stanford University School of Medicine, Stanford, California 94305.

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INDEX TERMS: Injury current • Diltiazem • Ventricular fibrillation • Myocardial ischemia

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REDUCTION OF ISCHEMIC DEPOLARIZATION
BY DILTIAZEM: A FLOW-INDEPENDENT EFFECT 883

William T. Clusin, Maurice Buchbinder, Avery K. Ellis,
Robert S. Kernoff, John C. Giacomini, Donald C. Harrison
Cardiology Division; Stanford U; Stanford CA

Ischemic depolarization and arrhythmias may result from effects of Ca overload on membrane permeability. We therefore compared the effects of diltiazem (D) on ischemic injury potentials and ventricular fibrillation (VF) during serial coronary artery occlusions in dogs. Injury potentials and VF were elicited every 15 min by simultaneous occlusion of the LAD and circumflex arteries during rapid atrial pacing. DC epicardial electrograms were recorded differentially between the ischemic zone and the distribution of a non-occluded proximal branch of the LAD. Injury potentials developed with a uniform time course during 5 control trials, but were reduced by D infusion (0.5-mg/kg) in 8 dogs. D reduced the mean T-Q depression at 150 sec of ischemia from 9.1 ± 2.7 mV to 6.1 ± 1.6 mV ($P < 0.001$). D increased the mean time from coronary occlusion to VF from 186 to 366 sec ($P < 10^{-5}$), but did not change T-Q depression at VF onset. D also delayed ischemia-induced conduction impairment to the same extent as T-Q depression. In 5 dogs, the effect of D on blood flow near the epicardial electrodes was measured using radioactive microspheres. Coronary occlusion reduced flow to the ischemic zone from 0.86 to 0.05 cc/min/g ($P = 0.001$). D increased pre-occlusion flow by 11% ($P = 0.03$), but did not significantly alter flow during occlusion. D did not diminish cardiac work. D therefore produced a flow-independent reduction of cellular depolarization during ischemia, which may reflect reduction of Ca overload.

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EFFECTS OF INCREASED RIGHT VENTRICULAR PRESSURE ON CORONARY BLOOD FLOW DISTRIBUTION. Avery K. Ellis*, John C. Giacomini, Robert Kernoff*, Donald C. Harrison. Stanford University Stanford CA 94305.

We studied the effects of acute changes in right ventricular systolic pressure (RVSP) on coronary blood flow distribution in ten open-chest mongrel dogs. Measurements were made using radioactive microspheres at three levels of RVSP: control, 31 ± 1.5 (SEM) mmHg; moderate RVSP elevation, 55 ± 2.0 mmHg, produced by constriction of the main pulmonary artery (MPA); and marked RVSP elevation, 80 ± 3.5 mmHg, produced by further MPA constriction. With intact autoregulation basal flow to the RV free wall averaged 0.76 ± 0.10 ml/min. The ratio of endocardial flow to epicardial flow (endo-epi ratio) was 1.05 ± 0.06 , and both overall flow and endo-epi ratio remained unchanged with increased RVSP. With maximal coronary vasodilation produced by carbocromen (5 mg/kg), overall RV flow increased to 4.54 ± 0.49 ml/min/g. The endo-epi ratio averaged 0.98 ± 0.08 with maximal vasodilation. At control RVSP, 0.91 ± 0.06 with moderate RVSP elevation, and 0.70 ± 0.06 ($p < 0.01$) with marked RVSP elevation. We conclude that, with intact autoregulation, normal coronary reserve mechanisms can adequately maintain RV subendocardial flow despite a marked increase in systolic compressive force. With maximal vasodilation, however, local flow reflects the interaction of driving pressure and local compressive force and elevations in RVSP result in a diminution of RV subendocardial blood flow. (Supported in part by NASA NCC2-126.)

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